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Chemical characterization, nematicidal and antioxidant activities of *Thymus linearis* Benth

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Abstract: *Thymus linearis* and its essential oil (EO) are used to cure a range of diseases in traditional medicine. GC-MS analysis of *Thymus linearis* EO revealed the presence of sixty-four components. Thymol (50.62 %), carvacrol (13.23 %), carvacrol acetate (7.72 %), β-bisabolene (5.47%), and o-cymene (5.47%) are the only five basic constituents in the oil which accounts for 82.07% of oil. When compared to other compounds, the EO and its primary components thymol and carvacrol had the largest proportion of mortality in *Meloidogyne javanica.* Carvacrol has also been proven to be helpful in suppressing the hatching of *M. javanica* eggs. This is the first time *T. linearis* EO and its constituents, such as borneol and caryophyllene, have been studied for nematicidal action. The antioxidant activity of EO components and active compounds was assessed using the ABTS radical scavenging method. Thymol and carvacrol were found to exhibit high antioxidant activity. The IC_{50} of thymol and carvacrol are found to be 38.18 g/ml and 49.65 g/ml, respectively, and are comparable to the positive control trolox (47.12 g/ml). Results clearly showed high potency for EO and its constituents, thymol and carvacrol as nematicidal and antioxidant agents.

Keywords: *Thymus linearis*, constituents, nematicidal activity, *Meloidogyne javanica*, antioxidant activity, ABTS

INTRODUCTION

Several herbs and their essential oils (EO) have been utilized as traditional treatments and are fundamental in aromatherapy since ancient times. Essential oils are complex combinations of volatile odoriferous secondary metabolites with anti-inflammatory, antibacterial, insecticidal, nematicidal, antioxidant, and anti-carcinogenic properties (Pandey *et al.,* 2014, Patil *et al.*, 2021; Chen *et al*., 2021; Mseddi *et al*., 2022).

Essential oils are produced by only a few plant families, most notably the Lamiaceae (Joshi *et al.,* 2016: Bashir *et al*., 2019). Plants belonging to the Lamiaceae are known to be rich in compounds possessing strong insecticidal, antimicrobial and antioxidant activities. The genus *Thymus* of Lamiaceae, which is known in vernacular as thyme, contains approximately 350 species of perennial, aromatic herbs and shrubs. It is widely distributed in the Mediterranean region, Asia, Southern Europe and North Africa (Hussain *et al.,* 2013; Naz *et al.,* 2015: Ahmed *et al.,* 2023). In traditional system of medicine aerial parts of *T. linearis* have been extensively used as herbal tea, antitussive, tonic and antiseptic agent (Rashid *et al.,* 2017; Bashir *et al.,* 2019; Ahmed *et al.,* 2023). It is used to treat high blood pressure, asthma, toothaches, headaches, colds, fevers, hazy vision, menstruation issues, eczema, psoriasis, and skin, eye, and liver illnesses (Akhtar *et al.,* 2014; Kumar *et al.,*2020). Thyme essential oil has been one of the top ten essential oils used in food preservation since ancient times (Kumar and coworkers, 2020). The phytochemical examination revealed that the plant extract contains a diverse range of phytochemicals, including reducing sugars, cardiac glycosides, phenolic compounds, flavonoids, and alkaloids (Naz *et al.,* 2015).

Thymol, *p*-cymene, borneol, carvacrol, thymol methyl ether, and phenolic mono-terpenes are the main chemical ingredients of *T. linearis* oil (Qadir *et al.,* 2016). *T linearis* has been shown to have powerful biological and pharmacological properties such as antioxidant (Jain *et al*., 2022: Chandra *et al*., 2016; Rashid *et al*., 2017), antibacterial (Naz *et al*., 2015; Kumar *et al*., 2020), antiproliferative (Hussain *et al*., 2013; Kumar *et al*., 2020), analgesic, antipyretic, anti-inflammatory (Qadir *et al*., 2016), antihypertensive (Akhtar *et al*., 2014) and vasorelaxant activities (Auger *et al*., 2018), making it one of the world's most popular herbs.

The major purpose of this study is to assess the biologically active chemical contents found in EO of *T. linearis* taken from the Hunza valley. This is the first report of nematicidal activity of *T. linearis* essential oil and its pure constituents borneol, thymol, carvacrol, and

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caryophyllene, against *Meloidogyne javanica*. This also describes the antioxidant potential of *T. linearis* essential oil and its active constituents in ABTS radical scavenging assay.

MATERIALS AND METHODS

Plant material

The aerial parts of T. linearis were collected in July and August from Karimabad, Hunza Valley, Gilgit-Baltistan, Pakistan, during the flowering and fruiting season. It was authenticated by Dr. Jan Alam, Department of Botany, Faculty of Science, Hazara University of Mansehra and dried under shady place. A voucher specimen (GH #: 94620) was deposited in herbarium of Department of Botany, University of Karachi.

Isolation of essential oil

The air dried aerial parts of *T. linearis* were subjected to steam distillation using a Clevenger apparatus. The first steam distillation was performed using total 1,616g of crushed aerial plant material in 2L distilled water for 6 hours and 4.6ml of yellowish essential oil was obtained (table 1). In second steam distillation 3,798g of aerial uncrushed plant material was used that afforded 17.7ml of yellow oil. In third distillation leaves and flowers (533.34g) were used, that provided 4.1ml of oil. The fourth distillation was carried out with 1008g of twigs to yield 2.1ml of yellow essential oil.

GC-MS (Gas chromatography-mass spectrometry)

Gas chromatography-mass spectrometry (GC-MS) analysis was performed using an Agilent GCMS single quad 5975. The EO was separated on a fused silica capillary HP-5 column, (length 30 m × diameter 0.25 mm) and with 0.25µm film thickness thick stationary phase film (5% Phenyl Methyl Siloxane). The flow rate of Helium carrier gas was 1 ml/min. The mass spectrometer was operated in the electron impact ionization (EI) mode at 70 eV in the scan range of 50-700 m/z. The split ratio was adjusted to 20:1 and the injected volume was 0.4µL. The injector temperature was set at 250°C and the oven temperature was kept at 60°C for 1min, rose to 180°C for 5°C $min^{-1}$and then increase to 300°C at 5°C min^{-1} and then hold for 15 min (total run time 72 min). Peak identification was performed by comparison with retention time of standards, and the mass spectra obtained were compared with those available in NIST libraries (NIST 05-Mass Spectral library, 2005 version) and Wiely-8 Mass spectral Library.

Nematicidal assay

Mortality test

Meloidogyne javanica egg masses were obtained from infected roots of greenhouse grown eggplants (*Solanumm elongenosa* L). Egg masses were pricked from the knots formed on the roots of infected egg plants using a fine needle and placed in sterile water. After hatching, number of juveniles were maintained around 40-60/ml in each cavity block 50µL of plant extract (10,000, 1000 and 500 ppm concentration in DMSO) was added in each cavity blocks containing 2 ml of sterile water. Three replicates of each treatment were made negative control was sterile water. Observations were taken after 24, 48 and 72 hours Nematodes were considered dead if they did not move when probed with a fine needle.

Hatching test

Eggs were extracted by using the modified method (McClure *et al*., 1973) using 2 % sodium hypochlorite solution. The eggs were washed under tap water using 100 (149µm pore size), 400 (38µm pore size) and 40µm mesh sieves. The residues collected on 400 and 40µm mesh sieve were separately transferred into distilled water forming egg suspension 1mL of egg suspension, 950µL of sterile water, 50µL of pure compounds/extract (for test) and 1mL of egg suspension and 950µL of sterile water (for control) were added in each cavity block and kept at room temperature with three replicates of each treatment.

Antioxidant assay

7mM solution of 2, 2-azino-bis (3-ethyl benzthiazoline-6-sulphonic acid) (ABTS) in 25ml EtOH was dissolved in 25ml of 25mM solution of potassium persulfate and left for incubation for 16 hours at ambient temperature in the darkness. Then dilute to obtain the absorbance (07±002) at 734nm. Stock solution (100ppm) of sample (10mg of sample in 100ml of ethanol) was prepared. Antioxidant property of the samples (05ml) was tested with the use of the UV spectrophotometer at different concentrations (100, 70, 50, 30, 10ppm) and 35ml ABTS solution. The absorbance was measured at 734nm after keeping six min in darkness (Badanai *et al*., 2015) Trolox was used as a positive standard. Percent inhibition was calculated by using following formula:

Inhibition [%] = $ABTS_{Abs}$-$sample_{Abs}$ × 100/$ABTS_{Abs}$

$ABTS_{Abs}$ = absorbance of the ABTS solution, $Sample_{Abs}$ = absorbance of the sample solution.

STATISTICAL ANALYSIS

For data analysis, each treatment was made in triplicate for the estimation of hatching and mortality activity. Data was expressed as mean ± error (Standard Error) and were analyzed by three way analysis of variance (ANOVA) using SPSS software (version 14).

RESULTS

The EO was subjected to GC-MS analysis, which showed the presence of sixty four constituents (table 2). Out of sixty four, fifty one compounds have been identified that constitutes 79.69% of total constitutes. Five of these are the major components (>5%) which constitute 82.07% of oil. It is worth mentioning here, that six constituents

bearing methyl isopropyl phenol nucleus including thymol, its isomer, carvacrol and their derivatives (ether and acetates), constitutes 76.63% of EO.

The dominating components of EO are thymol (50.62%), carvacrol (13.23%), carvacrol acetate (7.72%), β-bisabolene (5.47%), *o*-cymene (5.03 %), thymol methyl ether (2.33%), thymyl acetate (1.98%), 3-octanone (1.67%), caryophyllene (1.51%) and borneol (1.15%). The rest of components showed less than 1% quantity. The air dried aerial parts of *Thymus linearis* produced 0.47% of essential oil (EO) on steam distillation in Clevenger apparatus. However, maximum yield of EO in current studies (0.77%) is obtained from leaves and flowers. Twigs gave minimum yield of EO (0.21%). When production of EO was compared with crushed aerial parts it surprisingly gave lesser amount (0.29%) (*Vide* table 1). Literature however, showed larger amount of EO (0.8%) from plant grown in Quetta (Rashid *et al.*, 2017), 1.21% from Gilgit valley (Hussain *et al.*, 2013) and 2.62% from experimental field of CSIR Uttarkand, India (Kumar *et al*., 2020).

Nematicidal activity

The nematicidal activity of essential oil (EO) of *T. linearis* and its constituents revealed that EO, thymol and carvacrol have 100% mortality at the dose of 10,000ppm (100mg/ml) after 24 hours of treatment (table 4). Thymol at 1000ppm and carvacrol at 1000 and 500 ppm displayed comparable results after 72 hours of treatment. Thymol did not show any mortality at 500 ppm. Other constituents *i.e.* borneol and caryophyllene showed 39% and 23% mortality respectively after 72 hours at the dose of 10,000 ppm. The effect of EO and its constituents on egg hatching percentage of nematodes is more pronounced (table 5). All the tested compounds showed significant decrease in egg hatching percentage ranging from 1-10% after 72 hours at 10,000ppm except carvacrol which showed 0% egg hatching. Carvacrol exhibited comparable and maximum 5% egg hatching at 1000 and 500 ppm after 72 hours, while its isomer thymol is found less effective with 22% of egg hatching at 1000 ppm after same period of treatment. Caryophyllene and thymol displayed similar effects at 500 ppm.

Antioxidant activity

The antioxidant activity is described on the basis of the concentration providing 50% inhibition (IC_{50}). The lower IC_{50} value reflects high radical-scavenging activity. Trolox is used as a positive standard (table 6). The ABTS results of *T. linearis* are in good agreement with other methods like DPPH showing concentration depended rise in the scavenging activity of the EO. It showed minimum 6% inhibition at 10 µg/ml (ppm) while maximum inhibition was 55.59% at 100 ppm. Its IC_{50} is determined as 87.89µg/ml (ppm) which has trolox equivalence (TEAC) as 94.02µg/ml (table 6).

Thymol displayed 24.70% inhibition of ABTS at 10µg/ml while 89.13% inhibition was observed at 100µg/ml. Its IC_{50} is calculated as 38.17µg/ml which has trolox equivalent antioxidant concentration (TEAC) at 32.48 µg/ml. Carvacrol is the second major component of EO of *T linearis* that constitutes 13.23% of oil. It is positional isomer (5-isopropyl-2-methyl phenol) of thymol. In current antioxidant activity; it showed least inhibition 12.97% at 10µg/ml and maximal inhibition 90.11% at 100µg/ml. IC_{50} value of carvacrol has been determined as 49.65 µg/ml with trolox equivalence (TEAC) of 46.69µg/ml.

Linalool constitutes only 0.45 % of oil. It inhibits only 9.37% of radical at 10000µg/ml. Borneol constitutes 1.15 % of EO. However, it displayed poor antioxidant activity (4.15% of inhibition at 10000 µg/ml) when studied for the first time in current project.

DISCUSSION

The chemical composition of the EO of *T. linearis* is characterized by the presence of a higher amount of two positional isomers thymol and carvacrol. Several other researchers have also reported the presence of thymol& carvacrol with high percentages in EO (*vide* table 3) (Hussain *et al*., 2013,Verma, *et al.,* 2016, Rashid *et al.,* 2017., Kumar *et al*., 2020), and considered as the main components of EO in the family Lamiaceae (da Silva *et al*., 2021). However, the amount of thymol and carvacrol is highly varied in different regions. In Pakistan *T. linearis* is found in Hunza and Gilgit valleys of Baltistan and Quetta Balochistan. Quantity of thymol detected in current study is higher (50.62%) as compared to Gilgit valley (36.50%) and Quetta (10.19%). Amount of carvacrol is comparatively low in both Hunza (13.23%) and Gilgit (9.50%) (Hussain *et al.,* 2013). Nevertheless, the ratio of thymol and carvacrol was interestingly found similar i.e. 3.8: 1. When the ratio of thymol and carvacrol was compared with that of Quetta Baluchistan, it was surprisingly reversed i.e.1: 5.2. Carvacrol was found 52.88% while thymol was only 10.19% (table 3) (Rashid *et al*., 2017).On comparison with the Himalayan region of India highest percentage of thymol was found in Dhanachuli 66.80% (Chandra *et al*., 2016). However, ratio of thymol and carvacrol are much higher than found in Pakistan i.e. 36.6: 1 (Kumar *et al*., 2020), 63.1: 1 (Verma, *et al*., 2016) and 24.7: 1 in Dhanachuli (Chandra*et al*., 2016). It is interesting to note that EO obtained from Harinagar was completely devoid of thymol and carvacrol. Instead, it bears Germacrene (65.10%) and γ-Terpinene (19.41%) (Chandra *et al*., 2016).

In present study naturally grown plant from Hunza valley was steam distilled through Clevenger apparatus. Hence, variations in quantity of EO may be related to the plants growing in different region and mode of oil extraction.

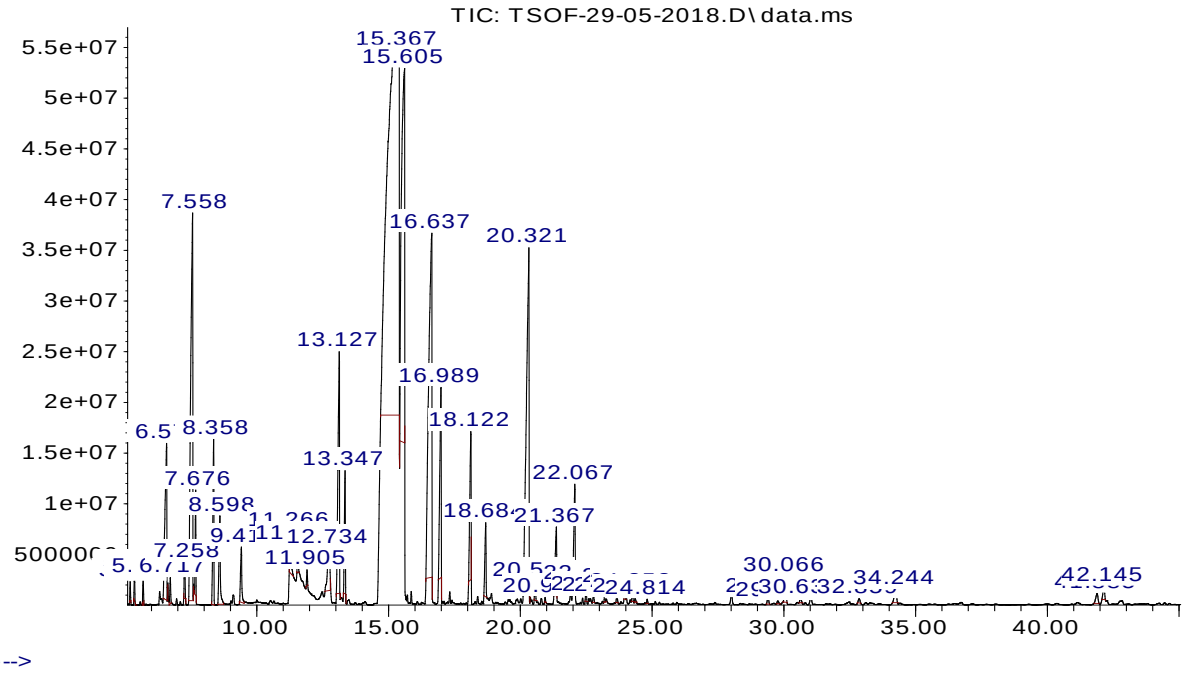

Fig 1: GC spectrum for essential oil of *T. linearis*

Table 1: Essential oil of twigs, leaves+flowers, crushed and uncrushed aerial parts of*Thymus linearis*

S No	Source	Essential Oil			Literature % (w/w)
		Volume (mL)	Weight (g)	% (w/w)	
1	Uncrushed aerial parts	17.70	17.75	0.47	2.26 (Kumar *et al*., 2020)
2	Crushed aerial parts	4.60	4.62	0.29	1.12 (Hussain *et al*.,2013) 0.87 (Rashid *et al*., 2017)
3	Leaves and flowers	4.10	4.11	0.77	Not reported
4	Twigs	2.10	2.11	0.21	Not reported

Table 2: The chemical constituents of essential oil of *Thymus linearis*

S. No	Compounds	Molecular formula	Molecular weight	Percentage Area %	Retention time	Kovats calculated (RI[d])	NIST (RI[e])
1	Unknown	-	-	0.07	5.186	-	-
2	α-Pinene[a,b]	$C_{10}H_{16}$	136	0.11	5.343	944	944
3	2,4 (10)-Thujadiene[a,b]	$C_{10}H_{14}$	134	0.01	5.578	952	958
4	Camphene[a,b]	$C_{10}H_{16}$	136	0.10	5.673	955	955
5	(-)-β-Pinene[a,b]	$C_{10}H_{16}$	136	0.01	6.310	977	977
6	3-Octanone[a,b]	$C_8H_{16}O$	128	1.67	6.515	984	985
7	Myrcene[a,b]	$C_{10}H_{16}$	136	0.06	6.588	987	987
8	3-Octanol[a,b]	$C_8H_{18}O$	130	0.11	6.683	990	992
9	α –Phellandrene[a,b]	$C_{10}H_{16}$	136	0.02	6.947	999	999
10	(+)-3-Carene[a,b]	$C_{10}H_{16}$	136	0.01	7.079	1004	1005
11	α-Terpinene[a,b]	$C_{10}H_{16}$	136	0.23	7.240	1010	1012
12	*o*-Cymene[a,b]	$C_{10}H_{14}$	134	5.03	7.489	1018	1018
13	β-Terpinylacetate[a,b]	$C_{12}H_{20}O_2$	196	0.05	7.562	1021	1267
14	Limonene oxide[a]	$C_{10}H_{18}O$	154	0.40	7.628	1023	-
15	γ-Terpinene[a,b]	$C_{10}H_{16}$	136	0.97	8.323	1047	1050

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16	Cis-β-Terpineol[a,b]	$C_{10}H_{18}O$	154	0.67	8.558	1055	1101
17	3-Nonanone[a,b]	$C_9H_{18}O$	142	0.01	9.004	1071	1064
18	Terpineolacetate[a,b]	$C_{12}H_{20}O_2$	196	0.05	9.092	1074	1336
19	Linalool[a,b,c]	$C_{10}H_{18}O$	154	0.45	9.378	1084	1080
20	Borneol [c]	$C_{10}H_{18}O$	154	1.15	11.245	1156	1163
21	(-)-4-Terpineol[a,b]	$C_{10}H_{18}O$	154	0.39	11.538	1168	1160
22	4-Terpineol[a,b]	$C_{10}H_{18}O$	154	0.03	11.728	1176	1176
23	α-Terpineol[a,b]	$C_{10}H_{18}O$	154	0.21	11.889	1182	1184
24	*p*-Cymen-8-ol[a,b]	$C_{10}H_{14}O$	150	0.09	12.036	1188	1189
25	Terpineol [a]	$C_{10}H_{18}O$	154	0.75	12.263	1197	-
26	2-Allyl-4-methylphenol [a]	$C_{10}H_{12}O$	148	0.06	12.585	1208	-
27	Thymol methyl ether[a,b]	$C_{11}H_{16}O$	164	2.33	13.057	1224	1235
28	Isothymol methyl ether[a,b]	$C_{11}H_{16}O$	164	0.75	13.302	1232	1244
29	Thymol[a,b ,c]	$C_{10}H_{14}O$	150	50.62	15.045	1289	1290
30	Carvacrol[a,b ,c]	$C_{10}H_{14}O$	150	13.23	15.257	1296	1298
31	Carvacrolacetate[a,b]	$C_{12}H_{16}O$	192	7.72	16.407	1343	1381
32	Thymolacetate[a,b]	$C_{12}H_{16}O$	192	1.98	16.817	1360	1352
33	(+)-3-Careneacetylmethyl [a]	$C_{13}H_{20}O$	192	0.04	17.220	1376	-
34	Unknown	-	-	0.01	17.300	-	-
35	Caryophyllene[a,b,c]	$C_{15}H_{24}$	204	1.51	18.025	1408	1408
36	Bicyclo[4.3.0]nonan-2-one, 8-Isopropylidene [a]	$C_{12}H_{18}O$	178	0.01	18.215	1415	-
37	α –Bergamotene[a,b]	$C_{15}H_{24}$	204	0.03	18.325	1419	1436
38	Naphthalene[a,b]	$C_{15}H_{24}$	204	0.02	18.486	1425	1209
39	4-tertbutylpyrocatechol[a,b]	$C_{10}H_{14}O_2$	166	0.27	18.581	1428	1493
40	α –Caryophyllene[a,b]	$C_{15}H_{24}$	204	0.06	18.838	1438	1438
41	β –Bisabolene[a,b]	$C_{15}H_{24}$	204	5.47	20.207	1487	1509
42	β –sesquiphellandrene[a,b]	$C_{15}H_{24}$	204	0.10	20.492	1497	1519
43	Cis-α-Bisabolene[a,b]	$C_{15}H_{24}$	204	0.03	20.902	1516	1511
44	4-methoxy-2,3,6-trimethylphenol [a]	$C_{10}H_{14}O_2$	166	0.56	21.276	1534	-
45	Unknown	-	-	0.06	21.832	-	-
46	Unknown	-	-	0.97	21.984	-	-
47	Ledene oxide- (II) [a]	$C_{15}H_{24}O$	220	0.03	22.323	1584	1631
48	Sapthulenol[a]	$C_{15}H_{24}O$	220	0.10	22.455	1590	-
49	β-Caryophyllene oxide [a,b]	$C_{15}H_{24}O$	220	0.02	22.565	1595	1596
50	Trans-longipinocarveol[a,b]	$C_{15}H_{24}O$	220	0.02	22.630	1599	1618
51	*Cis*-lanceol[a]	$C_{15}H_{24}O$	220	0.05	22.740	1603	-
52	Unknown	-	-	0.03	23.165	-	-
53	Unknown	-	-	0.02	23.230	-	-
54	Phytone	$C_{18}H_{36}O$	268	0.09	27.997	1841	1845
55	Unknown	-	-	0.06	29.396	-	-
56	Unknown	-	-	0.26	30.040	-	-
57	Unknown	-	-	0.06	30.626	-	-
58	Unknown	-	-	0.07	31.007	-	-
59	Hexadecanoicacid[a,b]	$C_{16}H_{32}O_2$	256	0.03	32.317	2045	1975
60	Unknown	-	-	0.06	32.845	-	-
61	Unknown	-	-	0.04	33.240	-	-
62	Unknown	-	-	0.17	34.192	-	-
63	Retinoic acid [a]	$C_{20}H_{28}O_2$	300	0.12	41.827	1885	-
64	Vitamin A acid[a]	$C_{20}H_{28}O_2$	300	0.18	42.082	1889	-

*Order of elution is given for column (HP-5); [a] Mass spectra were compared with literature fragmentation pattern given in National Institute of Standard Reference Data base Number 69; [b]Mass spectra and retention indices comparable with values given in literature available in NIST database;[c]Identification based on comparison of co-injected with standards;RI[d] calculated retention index; RI[e] literature retention index.

Table 3: Comparison of *Thymus linearis* constituents in different regions of Pakistan and India

S.NO	Compounds	Hunza (Natural)	Uttarakhand (Cultivated)	Gilgit (Cultivated)	Quetta (Natural)	Uttarakhand (Cultivated)	Harinagar (Natural)	Dhanachuli (Natural)
		Current Study 2020	Kumar *et al.,* 2020	Hussain *et al.,* 2013	Rashid *et al.,* 2017	Verma *et al.,* 2016	Chandra *et al.,* 2016	
	Monoterpene hydrocarbons							
1	α-Pinene	0.11	0.70	0.01	0.53	2.10	-	-
2	α-Terpinene	0.23	2.60	-	-	2.40	-	-
3	Camphene	0.10	0.40	0.59	0.63	0.30	-	-
4	Sabinene	-	0.10	0.06	-	-	-	-
5	β -pinene	0.01	0.20	0.69	-	-	-	-
6	Myrcene	0.06	1.80	-	0.80	1.60	-	-
7	α -Phellandrene	0.02	0.30	0.15	-	0.10	-	-
8	2-carene	-	-	1.38	-	-	-	-
9	(+)-3-Carene	0.01	0.10	0.09	-	-	-	-
10	o-Cymene	5.03	-	0.11	-	-	-	-
11	*p*-Cymene	-	5.20	3.46	2.92	13.10	-	9.80
12	β -Ocimene	-	-	0.01	-	-	-	-
13	γ-Terpinene	0.97	16.60	2.43	2.65	25.10	19.40	2.00
14	Limonene	-	0.40	-	-	0.10	-	-
15	Tricyclene	-	< 0.05	-	-	-	-	-
16	Cis-Thujone	-	< 0.05	-	-	-	-	-
17	α-Thujene	-	1.60	-	-	-	-	-
18	Terpinolene	-	-	-	-	< 0.05	-	-
19	Naphthalene	0.02	-	-	-	-	-	-
20	2,4 (10)-Thujadiene	0.01	-	-	-	-	-	-
	Oxygenated monoterpenes							
21	1,8-cineol	-	-	0.59	-	-	-	-
22	Cis-Sabinene hydrate	-	0.90	0.76	-	-	-	-
23	Terpineol acetate	0.05	-	-	-	-	-	-
24	Linalool oxide	-	-	0.06	-	-	-	-
25	Linalool	0.45	0.20	0.65	1.30	-	-	-
26	Isoborneol	-	-	2.04	2.48	-	-	-
27	Borneol	1.15	1.10	3.03	-	1.70	-	-
28	Terpineol	0.75	-	-	-	-	-	-
29	Terpinen-4-ol	0.03	0.30	0.90	1.49	2.50	-	-
30	α-Terpineol	0.21	0.10	1.46	-	-	-	-
31	Nerol	-	-	0.20	-	-	-	-
32	Piperitol	-	-	0.12	-	-	-	-
33	β-citronellol	-	-	0.05	-	-	-	-
34	Thymol methyl ether	2.33	3.20	4.03	-	1.20	-	2.00
35	Isothymol methyl ether	0.75	-	-	-	-	-	-
36	Thymol	50.62	54.90	36.5	10.19	44.20	-	66.80
37	Carvacrol	13.23	1.50	9.50	52.88	0.70	-	2.70
38	Carvacrol methyl ether	-	< 0.05	-	-	0.40	-	-
39	Carvacrol acetate	7.72	< 0.05	-	3.35	-	-	-
40	Thymol acetate	1.98	2.80	7.30	2.05	< 0.05	-	-
41	Geranyl acetate	-	-	1.51	-	-	-	-
42	Limonene oxide	0.40	-	-	-	-	-	-
43	Cis-β-Terpineol	0.67	-	-	-	-	-	-
44	(-)-4-Terpineol	0.39	-	-	-	-	-	-
45	*p*-Cymen-8-ol	0.09	< 0.05	-	-	1.40	-	-
46	(+)-3-Carene acetyl methyl	0.04	-	-	-	-	-	-
47	Bornyl acetate	-	0.10	-	-	-	-	-

Continue…

	Sesquiterpene hydrocarbons							
48	Caryophyllene	1.51	2.00	5.76	-	< 0.05	4.40	2.10
49	Aromadendrene	-	< 0.05	0.16	-	-	-	-
50	Amorphene	-	-	0.09	-	-	-	-
51	α -Caryophyllene	0.06	0.10	0.32	-	-	2.90	-
52	Allo-Aromadendrene	-	< 0.05	0.15	-	-	-	-
53	γ-muurolene	-	-	0.12	-	-	-	-
54	Germacrene D	-	0.20	0.22	-	-	65.10	3.90
55	β -Ionone	-	-	0.15	-	-	-	-
56	α-farnesene	-	-	0.56	-	-	-	-
57	β -Bisabolene	5.47	2.30	1.98	1.15	1.90	-	-
58	Calamenene	-	-	0.35	-	-	-	-
59	α -Bergamotene	0.03	-	-	-	-	-	-
60	β –sesquiphellandrene	0.10	-	-	-	-	-	-
61	Cis-α-Bisabolene	0.02	-	-	-	-	-	-
62	γ-Cadinene	-	0.10	-	-	-	1.80	-
	Oxygenated sesquiterpenes							
63	α-elemol	-	-	0.06	-	-	-	-
64	Caryophyllene oxide	0.02	< 0.05	3.78	-	0.20	-	-
65	Sapthulenol	0.10	< 0.05	0.10	-	< 0.05	-	-
66	Cadrol	-	-	1.39	-	-	-	-
67	α-muurolol	-	-	0.09	-	-	-	-
68	α-cadinol	-	-	0.24	-	-	-	-
69	Cis-lanceol	0.05	-	-	-	-	-	-
70	Trans-longipinocarveol	0.02	-	-	-	-	-	-
71	Ledene oxide- (II)	0.03	-	-	-	-	-	-
	Ketones							
72	3-Octanone	1.67	< 0.05	2.68	1.21	-	-	-
73	2-nonanone	-	-	0.07	-	-	-	-
74	3-Nonanone	0.01	-	-	-	-	-	-
75	Bicyclo[4.3.0]nonan-2-one, 8-Isopropylidene	0.01	-	-	-	-	-	-
	Phenolic compounds							
76	2-Allyl-4-methylphenol	0.06	-	-	-	-	-	-
77	4-tert butyl pyrocatechol	0.27	-	-	-	-	-	-
78	4-methoxy-2,3,6-trimethylphenol	0.56	-	-	-	-	-	-
	Alcohols							
79	3-Octanol	0.11	-	0.52	-	< 0.05	-	-
80	1-Octen-3-ol	-	-	-	-	0.10	-	-
	Others							
81	Hexadecanoic acid	0.03	-	-	-	-	-	-
82	β-terpinyl acetate	0.05	-	-	-	-	-	-
83	Retinoic acid	0.12	-	-	-	-	-	-
84	Vitamin A acid	0.18	-	-	-	-	-	-
85	Phytone	0.09	-	-	-	-	-	-
86	(E)-β-Ocimene	-	0.10	-	-	-	-	-
87	Methyl benzene	-	-	-	15.08	-	-	-
88	Cis-Thujone	-	< 0.05	-	-	-	-	-
89	Octanol acetate	-	< 0.05	-	-	-	-	-
90	Terpinolene	-	0.1	-	-	-	-	-
91	Thymol methyl oxide	-	-	-	0.16	-	-	-
92	Trans-Sabinene hydrate	-	< 0.05	-	-	< 0.05	-	-
93	Viridiflorene	-	< 0.05	-	-	-	-	-

Table 4: Effect of Essential Oil of *Thymus linearis* and Its Constituents on *Meloidogyne javanica* Mortality

Treatments (ppm)		Juvenile's mortality (%)		
	Doses (ppm)	24 hours	48 hours	72 hours
EO	10,000	100	100	100
Borneol	10,000	3±3.33	7±6.66	39±6.75
	1000	0±0	0±0	4±4.16
	500	0±0	0±0	11±1.11
Thymol	10,000	100±0	100±0	100±0
	1000	0±0	11±1.11	22±2.22
	500	0±0	0±0	0±0
Caryophyllene	10,000	0±0	18±9.68	23±5.64
	1000	0±0	0±0	20±7.69
	500	0±0	0±0	7±6.66
Carvacrol	10,000	100±0	100±0	100±0
	1000	3±3.33	4±4.16	21±3.21
	500	4±4.16	12±6.18	27±11.76
Positive control (DMSO+ nematode)	-	0±0	0±0	5±5.55
Negative control (water+ nematode)	-	0±0	0±0	0±0

$LSD_{0.05}$ Concentration = 3.96; Treatment = 3.54; Time = 3.07 : Data was expressed as mean ± error (Standard Error) and were analyzed by three way analysis of variance (ANOVA)

Table 5: Effect of Essential oil of *Thymus linearis* and its constituents on egg hatching of *Meloidogyne javanica*

		Egg hatching (%)		
Treatments (ppm)	Doses (ppm)	24 hours	48 hours	72 hours
EO	10,000	1	1	1
Borneol	10,000	0±0	7±2.43	9±3.62
	1000	1±1.38	9±3.13	16±6.13
	500	3±1.73	29±1.94	11±3.07
Thymol	10,000	1±1.33	3±1.26	4±2.31
	1000	4±2.40	14±6.05	22±7.62
	500	6±0.36	7±0.60	15±1.84
Caryophyllene	10,000	0±0	4±1.96	10±0.71
	1000	5±1.33	6±1.04	13±0.51
	500	6±0.78	7±1.51	15±0.72
Cravacrol	10,000	0±0	0±0	0±0
	1000	2±1.03	2±1.03	4±0.89
	500	2±1.96	3±3.33	5±2.91
Positive control (DMSO+ nematode)	-	2±1.66	9±1.04	20±2.26
Negative control (water nematode)	-	15±4.12	22±4.57	42±6.35

$LSD_{0.05}$ Concentrations = 3.23; Treatment = 2.89; Time = 2.50: Data was expressed as mean ± error (Standard Error) and were analyzed by three way analysis of variance (ANOVA)

Table 6: TEAC and IC_{50} values of Compounds and EO of *Thymus linearis in ABTS Cation De-Colourization Assay*

SNO	Compounds	IC_{50} Values (µg/mL)	TEAC µg/mL
1	EO	87.9	94.0
2	Thymol	38.2	32.4
3	Carvacrol	49.7	46.6
4	Borneol	< 100	ND
5	Linalool	< 100	ND

Literature revealed that uncrushed plant grown for research purpose and hydrodistilation produced better yield of EO as compared to steam distillation and naturally grown plant. In Pakistan plant from Gilgit valley appears to provide larger amount of EO as compared to plant from Hunza (current work) and Quetta (Rashid *et al.*, 2017). The chemical profile of current work was found to be in good agreement with plant in Gilgit valley,

which also reported the thymol, carvacrol, thymol acetate and caryophyllene as major constituents of EO, but the amount of compounds showed variations in their percentages. This may be related to the naturally grown sample of *Thymus linearis* in Hunza valley while the samples from Gilgit valley are actually cultivars (Hussain *et al.,* 2013).Among others major constituents of EO, thymol acetate was detected in most of the species (*Vide* table 3) while carvacrol acetate was detected only in current study, Quetta (Rashid *et al*., 2017) and Uttararakhand (Chandra*et al*., 2016). Thymol methyl ether was detected in most of them while carvacrol methyl ether was observed in Uttararakhand only (Chandra *et al*., 2016: Verma*et al*., 2016). However,sixty four constituent of EO have been detected in current study as compared to literature which include forty eightinGilgat (Hussain *et al*., 2013), forty two in Utterkand (Kumar *et al*., 2019), twenty five in Utterkand (Verma *et al*., 2016), sixteen in Quetta (Rashid *et al*., 2017), five in Harinagar and seven in Dhanachuli (Chandra *et al*., 2016).

Percentage of thymol present in *T. Linearis* varies regionally from (66.8% - 10.19%), while that of carvacrol ranges from (52.8% - 0.7%). Higher amount of carvacrol acetate (7.72%) is observed in current investigation (*vide* table 3) and not found in neighboring Gilgit valley (Hussain *et al*., 2013). This study also revealed the difference of constituents between the naturally grown plants and those cultivated in research field. Out of seven, five plants showed the existence of β-bisabolene with (5.47% - 1.15%). The two Himalayan valleys show its extremities (*Vide* table 3). *O*-cymene is observed only in Hunza (current) and Gilgit valley (Hussain *et al*., 2013) while its isomer *p*-cymene has been identified in addition to Hunza and Harinager. The EOs from Quetta region is devoid of β-caryophyllene. The variation in EO constituents of *T. linearis* in different regions may be attributed to the environmental factors, soil condition and mode of extraction.

Root knot nematodes (RKNs) are one of the most destructive nematodes. They disturb the normal function of plant by gall formation on root that obstructs water and nutrient supply which causes weakness of plant and ultimate death. They severely damage a wide range of agricultural crops and serious yield losses worldwide (Hemmati *et al.,* 2019). Although, chemical nematicides have been used as one of the primary means for controlling RKNs, reliance on these nematicides is associated with heavy costs and negative effects on human health and environment (Cayrol *et al.,* 1989; Tariq *et al.,* 2007).Thus, the use of non-chemical management strategies is recommended to avoid such disadvantages. Plant essential oils bear insecticidal activity and have ability to selectively kill/degrade parasites and their toxic products with little harmful effect on non-target organisms and environment (Hemmati *et al.,* 2019; Oka et al., 2000). On the basis of current results, it may therefore be concluded that carvacrol is more significant to inhibit the *M. javanica* eggs, while its isomer thymol and EO itself have highest rate of mortality as compared to other constituents. The nematicidal activity of thymol and carvacrol has been reported in literature (Nikolić *et al.,* 2014; Galovičová *et al*., 2021). Both thymol and carvacrol showed effective inhibiting activity at a concentration of 250µl/L under diverse experimental conditions.

Antioxidants are the substances which can scavenge free radicals and prevent and repair damages, therefore, can decrease the risk of cancer, diabetes, cardiovascular and other degenerative diseases. Nowadays interest in natural antioxidants has also been increased in food industry due to possible toxic effects of synthetic antioxidants (Lee *et al.,* 2015). Various methods like DPPH, ABTS, FRAP etc have been used to determine the antioxidant activity of natural substances (El Abed *et al.,* 2014). 2, 2′-azino-bis-3-ethyl benzothiazoline-6-sulphonic acid (ABTS) antioxidant activity is one of the most popularly used methods. This probably is due to the fact that ABTS is a stable chromogen to measure the antioxidant activity of biological material in a relatively short time period as compared to other methods (Stobiecka *et al.,* 2014). The ABTS assay is complementary colorimetric method. This scavenging radical method is based on a reduction of the blue-green radical cation of ABTS by electron donors. As a result, the strong absorption of ABTS radical cation (ABTS•+) that is observed in the range of 600-750 nm disappears, and the free radical scavenging activity of the investigated compound can be directly evaluated from the percentage of ABTS inhibition (Rúa *et al.,* 2019). It is interesting to mention here, that reported IC50 of EO of T. vulgaris and T. Serpyllumare 0.30 g/L (300µg/ml) and 0.40g/L (400µg/ml) respectively in DPPH antioxidant assay (Lee *et al*., 2015).

Literature however, revealed different IC_{50} of thymol like 140µg/ml (Sharopov *et al*., 2015) and 1.7µg/ml (da Silva *et al*., 2021). Difference in IC_{50}values may be due to different wave length and incubation period used during assay. In present studies wave length at which absorbance recorded was 734nm (Verma *et al*., 2016) while in literature it is 645 nm. Incubation period in current study is 6min. as compared to 30min. in literature (Miguel *et al*., 2009; Yildiz *et al.,* 2021). Literature however, revealed its IC_{50} value as 21µg/ml, which may be correlated to the change in wave length (645nm) and time difference in incubation period (Sharopov *et al.,* 2015).

Thymol (2-Isopropyl-5-methylphenol) is 50.62% of the EO of *T. linearis.* It has phenolic nucleus and has been registered by European commission for its use as a flavoring agent in food stuffs at low concentration

(Ceylan *et al*., 2016). Phenolic compounds are mainly responsible for the antibacterial and antifungal activities in *Thymus* species. As phenolic constituents are lipophilic in nature, their antibacterial and antifungal action is correlated with the damaging of their lipid membrane possibly due to the interference of phenolic components on the cell wall enzyme (chitinase) (Kumar *et al*., 2020; da Silva *et al*., 2021).

Carvacrol possesses antioxidant, anti-inflammatory, antitumor, analgesic and insecticidal properties (Miguel *et al.,* 2009). Like thymol, it is also used as a flavoring agent and food preservative. It possesses antimicrobial activity against bacterial, fungal spoilage and pathogenic food borne microorganisms (Rúa *et al*., 2019).

Linalool is used as fragrance ingredient in fancy perfume compositions of cosmetics. It is also used in detergents and cleaners for fragrance. It has been reported as antimicrobial agent. It is also considered as a potent anti-tumor agent as well as the lead molecule for the synthesis of new anticancer drugs (Stobiecka *et al.,* 2014). Linalool has already been reported as weak scavenger among the EO constituents (Stobiecka *et al*., 2014). Borneol is bicyclic monoterpenoid alcohol and used in food, cosmetics and traditional medicines to treat painful and inflammatory conditions. It is promising anti-inflammatory and antimicrobial agent (Silva *et al.,* 2016).

It can therefore be concluded that the thymol and carvacrol are the most significant antioxidant constituents of essential oil (EO) of *T. linearis*. IC_{50} of thymol is more significant (38.18 µg/ml) than positive standard trolox (47.12 µg/ml) while carvacrol (49.65 µg/ml) has antioxidant effect comparable to trolox.

CONCLUSION

The present study indicated that the essential oil (EO) extracted from naturally grown *T. linearis* of Hunza valley Pakistan, is rich in monoterpenes and oxygenated monoterpenes. EO and its major isomeric constituents, thymol and carvacrol bear remarkable nematicidal and antioxidant activities. Further studies on these constituents may transform these findings into potent bio-insecticide to replace conventional chemical pesticides.

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